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Grain protein under future climate conditions of elevated temperature and carbon dioxide. A study of 108 spring barley accessions

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Abbreviations: GPC: grain protein concentration; GPH: grain protein harvested; GWAS: Genome Wide Association Studies; MSC: multiplicative signal corrected; NIR: Near infrared radiation; PLSR: partial least squares regression; RERAF: Risø Environmental RiskAssessment Facility; RMSECV: root mean square error of cross validation; RMSEP: root mean square error of prediction; SEP: standard error of performance

Abstract

Climate change is predicted to decrease future grain yields and influence grain protein concentration. In the present study a set of 108 spring barley accessions were cultivated under predicted future levels of temperature, [CO_2] and [O_3] as single-factors and temperature and [CO_2] in combination (IPCC SRES scenario A1FI). The found 8 % increase in grain protein concentration under the combined treatment could not be depicted from the single factor treatments. Ozone as single factor increased grain protein concentration with 6 %. In a future scenario with projected lowered grain yield, harvesting as much protein as possible seems desirable. Grain protein harvested only increased under elevated [CO_2] and was lowered 23 % in the future climate scenario of elevated temperature and [CO_2]. Vast variation in the response of the 108 accessions was identified. This variation should be further exploited to increase the grain protein harvested under future climate change conditions.

Introduction

Climate change, with increased atmospheric concentration of the greenhouse gasses carbon dioxide ([CO_2]) and ozone ([O_3]) together with rising temperature, is likely to decrease plant production in the future and influence grain protein and quality (Danielsson *et al.*, 1999; Lobell and Field, 2007; Wang and Frei, 2011; Collins *et al.*, 2013; IPCC, 2014*a*). According to the latest projections by IPCC (Intergovernmental Panel of Climate Change) climatic conditions point to the worst-case scenario (RCP8.5) unless actions are taken in the near future (IPCC, 2014*b*). In the worst-case scenario temperature is predicted to rise 5 °C and [CO_2] to reach 1000 ppm compared to the 400 ppm of today. The increase of [O_3] is expected at 25 % reaching 40-77 ppb (Collins *et al.*, 2013; Ellermann *et al.*, 2013). Numerous experimental studies have demonstrated the effect on cereal grain yield by elevated temperature, [CO_2] and [O_3] as single- factors; increasing production by [CO_2] (Wang *et al.*, 2013) and decreasing production by temperature (Luo, 2011) and [O_3] (Feng and Kobayashi, 2009). Less studies have reported the effect on grain yield by the combination of climatic factors (Mittler, 2006; Frenck *et al.*, 2011; Pleijel and Uddling, 2012; Dias de Oliveira *et al.*, 2013). In studies of elevated temperature and [CO_2] combined, grain yield was found to decrease by 14-53 % (Batts *et al.*, 1998; Clausen *et al.*, 2011; Ingvordsen *et al.*, 2014). The decrease in cereal grain yield with a global temperature increase of > +3 ˚C is critical in the context of maintaining a sufficient primary production, which can meet the needs of a growing world population and improved living standards.

Grain protein concentration (GPC) has been reported to increase in response to abiotic stress such as heat, drought and elevated [O_3] (Savin and Nicolas, 1996; Passarella *et al.*, 2008; Asare *et al.*, 2011; Pleijel and Uddling, 2012), while GPC was decreased by elevated [CO_2] (Högy and Fangmeier, 2008). Timing of the climate effect in plant development was further found to influence the response in GPC (Rotundo and Westgate, 2009; Wang and Frei, 2011). Grain protein is decisive for several end-uses, however, the effects on GPC by combined climatic factors is little studied even though factors of climate change will appear concerted (Wang and Frei, 2011; Högy *et al.*, 2013).

The fourth major cereal of the world is barley (*Hordeum vulgare* L), which in temperate climates is cultivated predominantly as a spring crop for feed to livestock and malt for use in brewing and distilling industries. Barley has though, within the recent years, achieved increased attention for human consumption due to its high nutritional value and potential health benefits (Baik and Ullrich, 2008). The diverse uses of barley grains cause different demands to the grain composition. Generally, high protein content is preferable in barley for feed, whereas a low protein grain and high starch content is preferred for malting purposes. Climate change alterations in protein content can in the industrialized countries in the temperate zone also have substantial marked implications with economic and social consequences.

In the context of climate change with projected decreased grain yields (IPCC, 2014*a*), the grain protein harvested (GPH) is important for product quality and secured primary production. Few studies have focused on the impact of climate change on cereal grain protein determining for the quality and even fewer in the context of GPH in barley. The objective of this study was to examine climate change effects to an array of accessions. Here we present the effect on grain yield and GPC under the combination of elevated [CO_2] and temperature as under the single-factors elevated [CO_2], temperature and [O_3] on 108 spring barley accessions.

Material and methods

Plant material

One hundred and eight 2- or 6-rowed primarily Nordic spring barley (*Hordeum vulgare* L.) accessions were included in the study (Table 1). The set included 38 landrace accessions, 25 old cultivars (before 1975), 41 modern cultivars (after 1975) and four breeder-lines. Accessions were supplied by NordGen or Nordic barley breeding companies. For pedigree, breeder institute, and provider please see S1.

Experimental set up

Five climate treatments were applied in the RERAF phytotron (Risø Environmental Risk Assessment Facility) at the Technical University of Denmark, Roskilde (http://www.eco.kt.dtu.dk/Research/Research_Facilitites/RERAF) to all 108 accessions throughout their full lifecycle. The 108 accessions were a subset of the 138 accessions analysed by Ingvordsen et al. (2014) for quantity of production. Within each of the five 24 m^2 chambers (height 3m) in the phytotron, humidity, temperature and gasses were controlled as well as continuously monitored. The five applied treatments can be seen in Table 2. They included (1) ambient (control) mimicking present south Scandinavian summer of 19/12 °C (day/night), [CO_2] constantly at 385 ppm and no O_3 added, (2) [CO_2] constantly at 700 ppm, (3) temperature elevated +5 °C (day and night), (4) elevated temperature and [CO_2] combined at the level of the single-factor treatments and (5) [O_3] constantly at 100-150 ppb (day and night). The climatic factors were mimicking levels predicted ultimo 21st century, if greenhouse gasses are not substantially reduced (SCRES scenario A1FI, IPCC, 2007). The CO_2 was supplied by Air Liquide A/S Denmark and O_3 by UV Pro 550A generators (Crystal air products & services, Canada). Further details on RERAF are given by Frenck *et al*. (2011) and Ingvordsen *et al*. (2014). Eight plants of each accession were grown in 11 L pots with 4 kg of sphagnum substrate (Pindstrup Substrate No. 6, Pindstrup Mosebrug A/S, Denmark), where 10 g of NPK fertilizer (21-3-10, Yara) was applied at sowing. Water was applied within one hour after the light was turned on by a surface dripping system delivering 4.4 L m^{-2} day^{-1} in all treatments. To compensate for the drainage of the pot setup as well as root distribution and water loss, water was applied above the average precipitation of Southern Scandinavia (236 mm; DMI, 2014). Watering was stepwise reduced from Zadoks growth stage (ZGS) 90 and ended at ZGS 99 (Zadoks *et al*., 1974). Light was supplied by 28 high-pressure mercury (1000 W or 400 W) and 14 halogen (250 W) lamps in each chamber. The daily light cycle was 16/8 h (day/night) and PAR (parabolic aluminized reflector) averaged at approximately 400 mol photons m^{-2} s^{-1} at canopy height (ca. 1 m). To avoid possible chamber specific effects the treatments with its corresponding batch of plants were rotated between the chambers on a weekly basis. In practice all plants were exposed to ambient conditions for approximately 2 hours during the time of rotation and the time necessary for the new chamber to reach the different treatment values.

Grain yield

Plants were harvested individually and after drying in constant temperature for a minimum of three weeks, they were threshed and grain weight measured. After threshing grains were stored at 7 °C. Number of grains was obtained by dividing with the weight of an enumerated sub-sample.

Protein

Total nitrogen (N)-analyses and following calculation of crude protein were performed by YARA (Yara Analytical Services, Pocklington, England) on grain material via the Dumas Combustion method on a LECO CNS TRUMAC. Crude protein was achieved on 17 accessions (stated in S1) in each of the five treatments, and used to predict protein concentration in the remaining accessions.

NIR measurements

Spectral reflectance of whole grains from all accessions was obtained using a QFA-Flex 600F FT-NIR instrument (Q-interline, Tølløse, Denmark). 1.5-7 g of the grains were placed in IR transparent glass vials (height 6 cm, diameter 2.6 cm) and measured using a rotating sample device. The sample was rotated at three rounds per minute. The measuring sample window at the rotating sample device had a diameter of 6 mm, which provides an analysis surface of approximately 510 mm^2. Spectra were collected at every 2 nm in the NIR region from 1100 to 2498 nm. One spectrum was obtained for each sample as an average of 64 sub-scans. The spectra were reported as log (1/R).

Statistics

Principal component analysis (PCA) was performed on raw data as an explorative data analysis to obtain a first overview of the data and to identify obvious outliers and delineate classes. Hotelling's T-square versus residual plots was used to detect outliers. Partial least squares regression (PLSR) models were developed on raw scatter corrected spectra, Savitsky-Golay first derivative (Savitzky and Golay, 1964) averaging over 7 points and a second order polynomial spectra and multiplicative signal corrected (MSC) (Geladi, 1985) spectra. Root mean square error of cross validation (RMSECV) plotted against the number of PLSR latent variables for each pre-processing method was used to select the optimum pre-processing method and the optimum number of latent variables in the PLSR model. The optimum number of latent variables was chosen as the first local minimum in the smooth declining RMSECV curve or the point, where this curve flattened. Random cross validation with 10 segments and 10 iterations was used.

The performance of the PLSR model to predict protein were evaluated using the root mean square error of prediction (RMSEP), standard error of performance (SEP) and bias. Initially the obtained model was developed on 17 accessions per treatment and used to predict protein concentration in the remaining accessions.

All analysis were carried out using MATLAB version 7.9.0 (R2009b) (The Mathworks, Inc., Natick, MA, USA) along with the PLS toolbox version 7.5.1 (Eigenvector Research, Inc., Manson, WA, USA).

Following statistical analysis was carried out in R version 2.15.3(R core Team, 2013), and SigmaPlot version 11.0, (Systat Software, Inc., San Jose California USA, www.sigmaplot.com) was used for illustration.

Results and discussion

Quality of applied treatments

Atmospheric conditions of temperature and relative humidity were during cultivation in rather good agreement with set points programmed in the RERAF phytotron. With regard to experimental levels of [CO2] the difference between the treatment of ambient and elevated [CO2] was app. + 240 ppm and on average 75 ppm lower than expected (Table 2). The increased [CO2] in treatments with ambient levels of [CO2] is most probably due to that CO2 cannot technically be removed, and the large amount of plants seemed to have produced considerable quantities of CO2 during respiration.

Treatment effects on grain yield

The effects of the single climatic factors on overall grain yield of the 108 accessions were reported as a subset of 138 accessions accounted for in Ingvordsen et al. (2014). Grain yield was found in agreement with previous studies, reporting increased grain yield at elevated [CO_2] (Ziska and Bunce, 2007) and decreased grain yield at elevated [O_3] (Feng *et al.*, 2008) and elevated temperature (Barnabás *et al.*, 2008) as under the two-factor treatment of elevated temperature and [CO_2] combined (Clausen *et al.*, 2011).

NIR and prediction of GPC

A PLSR model based on NIR measurements and chemical measurement of N with subsequent calculation of GPC was developed and used to predict GPC in the remaining accessions. Spectra for all included accessions showed sufficient variance and clear peaks for further analysis (Fig. 1). A good calibration model using 8 latent variables on MSC pre-processed NIR spectra showed an R^2=0.8 with an RMSECV=1.34. Based on this calibration model the protein concentration was predicted in the remaining accessions (Fig. 2).

Treatment effects on GPC

Elevated temperature as single-factor caused GPC to increase 29 % (Table 3). Several studies have reported increased GPC from elevated temperature >35 °C (Savin and Nicolas, 1996; Majoul-Haddad *et al.*, 2013) or around anthesis (Pettersson and Eckersten, 2007; Malik *et al.*, 2013). In the present study, a constantly elevated temperature of +5 °C was also found to increase GPC. Högy *et al*. (2013) found no change in GPC from a 2 °C increase in soil temperature, but decreased concentrations of total non-structural carbohydrates, starch, fructose and raffinose. The increase in GPC appears promising in terms of securing sufficient protein production under future climate conditions, however, in a future climate [CO_2] is projected to increase concerted with temperature.

Under elevated [CO_2] the GPC decreased overall 5 % (Table 3). This was less than the 15 % decrease found in a meta-analysis of barley with no significant difference between FACE, open-top chambers and enclosure studies or if rooted in pots or field (Taub *et al.*, 2008). The less decrease induced by elevated [CO_2] found in the present study might be due to the plant material tested, as elevated levels of [CO_2] were in agreement (590-700 ppm). The material used in the meta-analysis presumably covered four barley cultivars (Thule, Alexis, Jo1621 and Atem) (Kleemola *et al.*, 1994; Thompson and Woodward, 1994; Sæbø and Mortensen, 1996; Fangmeier *et al.*, 2000), whereas the present study included 108 accessions (Table 1 and S1).

Elevated [O_3] was found to increase overall GPC with 6 % (Table 3). Studies in wheat, which has been reported more sensitive to [O_3] than barley (Mills *et al.*, 2007), have found GPC of wheat to increase overall 7 % with averaged [O_3] of 58 ppb and exposure between 7-12 hours per day (Feng *et al.*, 2008). The study by Feng et al. (2008) also reported 71 ppb [O_3] to cause further increased protein concentration. In the present study [O_3] averaged 121 ppb on a 24 hours basis. The similar increase in GPC from the double concentration and exposure-time to [O_3] may suggest that barley is not very sensitive to O_3 or that barley has a different responds pattern to ozone than wheat.

The 29 % increased GPC under elevated temperature was modified to 8 % under the simultaneous exposure to elevated temperature and [CO_2] in the two-factor treatment (Table 3). This result strongly points to the risk of misinterpretation of the combined effects, when deduced from single-factor treatments. The combined effect of elevated temperature and elevated [CO_2] on GPC was not found to be additive - an important point when considering the future effects of climate change, where temperature, [CO_2] and [O_3] are predicted to increase concerted.

Treatment effects on grain protein pr. grain

Considering the quantity of grain protein in relation to the weight of a single grain (Table 3), the picture changed from increase to decrease under elevated [O_3] and the two-factor treatment compared to response in GPC. Under elevated [CO_2] the GPC and protein per grain decreased similarly, 5 % (Table 3). The 29 % increase in GPC under elevated temperature was substantially lowered to only 7 % when given on a pr. single grain weight basis. The decreases reflected the diverse seed weights in the different treatments, where only the treatment with elevated [CO_2], had more or less the same seed weight as found under ambient conditions (data not shown). However, a suggested inhibition of the assimilation of nitrate into e.g. proteins under elevated [CO_2] (Bloom *et al.*, 2010) could have engaged in the maintained and not increased grain weight by elevated [CO_2]. Further, the increase in GPH was found to be smaller than the increase in grain yield as previously reported as indication on inhibition of the assimilation of nitrate under elevated [CO_2] (Pleijel and Uddling, 2012).

Treatment effects on GPH

The increased GPC in the two-factor treatment that could potentially increase protein production under future climate conditions vanished, when the treatment effect on actual harvested quantity was considered (Table 3). Even though the GPC increased 8 % compared to ambient, the GPH was found decreased by 23 % due to the decreased grain yield of 28 % in an atmosphere of elevated temperature plus [CO_2]. The treatments effects on grain yield converted the potential increase in GPH into an overall reduction, as was also seen in the single-factor treatments with elevated temperature and [O_3]. In the single-factor treatment with elevated [CO_2] the opposite was observed, as the decreased GPC was compensated for by the higher yield, and the resulting GPH was increased compared to ambient (Table 3).

Since the findings of the present study are based on 108 accessions the overall effects reported are considered robust with regard to barley, and the characteristics identified might be considered of value in future breeding. Responsiveness to the elevated [CO_2] has been suggested as a breeding target to increase grain yield under future climate conditions (Ziska and Bunce, 2007; Franzaring *et al.*, 2013; Ingvordsen *et al.*, 2014). In the present study we found that the GPC under elevated [CO_2] was decreased, though relatively little in comparison to the increased grain yield, suggesting that a substantial increase in GPH could be envisaged from improved CO_2-responsiveness. Harvested grain protein was found increased (13 %) under elevated [CO_2] - not from increased protein pr. grain but from increased production of grains (Table 3) (Jablonski *et al.*, 2002; Ingvordsen *et al.*, 2014). Application of additional nitrogen-fertilizer could potential ameliorate the loss of protein in the grain under elevated [CO_2], however, Bloom *et al.*(2014) reported an insignificant effect on GPC in wheat leaves under elevated [CO_2] due to inhibited nitrate assimilation. The suggested inhibition of protein accumulation by elevated [CO_2] requests better understanding of ammonium and nitrate use by crops under climate change conditions, an area that has received little attention (Andrews *et al.*, 2013).

Grain protein in the 108 accessions

Among the 108 accessions, some differed to a greater or lesser extent from the overall responses to the treatments, suggesting great diversity that could be exploited in breeding programs. No significant difference in response to the climate treatments were observed between the group of landrace and the group of cultivars (Fig. 3 and 4).

Considering the expected lower grain yield under future climate conditions harvesting as much protein as possible is likely preferable. Under the two-factor treatment the 108 accessions decreased in average 23 % in GPH relative to ambient, however, the individual accessions spanned from -60% to 30 % GPH (Fig. 3). Two landraces (Kushteki and Moscou) and a 2-rowed Danish feed barley cultivar (Jacinta) increased 30-33 % in GPH. All three accessions ranked in top ten for grain yield of the 108 accessions, whereas only the feed barley ranked in the top (placed 2) in GPC in the two-factor treatment. Another four accessions, two modern cultivars (Sebastian and Brage), a Finish landrace (Luusua) and a breeder-line (Bor 05135) increased 13-16 % in GPH under elevated temperature and [CO_2] in combination. Of these four accessions, only the landrace demonstrated high rank (8) with regard to GPC under the two-factor treatment of all 108 accessions, and the Danish cultivar (Brage) demonstrated high grain yield. The last two accessions, the Norwegian 6-rowed cultivar and the Finish 2-rowed breeder-line demonstrated top-medium rank for GPC and grain yield, where they ranked 52 and 21 in GPC and 22 and 14 in grain yield. When only considering the performance in the two-factor treatment, all seven accessions rank in top 15 of the 108 accessions in GPH. That increased GPH was identified in landraces, cultivars and a breeder-line as in 2- and 6-rowed suggest that beneficial genes for developing cultivars with high GPC and grain yield are available from many sources.

Under elevated [CO_2], three accessions increased over 80 % in GPH. The accessions were two 2-rowed old Swedish accessions (Arla and Pallas) and the 2-rowed Danish feed cultivar that also demonstrated increased grain yield under the two-factor treatment (Jacinta). In the pedigrees of both Jacinta and Arla the accession Bavaria (NGB6945) can be found(van Berloo and Hutten, 2005). In addition both Jacinta and Arla showed high CO_2-responsiveness, both among the top five accessions increasing most in grain yield under elevated [CO_2]. All three accessions had high grain yields under elevated [CO_2], whereas under ambient conditions the old Swedish cultivars ranked low (81 and 106) in GPC and grain yield (79 and 96).

Elevated temperature increased overall GPC the most and was only found decreased in eight accessions being landraces and old cultivars. Overall grain protein harvested was decreased by 42 % but three accessions showed increased GPH under elevated temperature, all 2-rowed and cultivars; an old Swedish (Mari), an old Danish (Odin) and a modern Danish (Sebastian).

One can speculate if the ability of Jacinta and Sebastian to produce high GPH in the two-factor treatment was related to their suggested improved ability to secure high GPH in either of the single-factor treatments of elevated [CO_2] or elevated temperature – or reverse; the performance in either of the single-factor treatments contributed to the performance under the combined treatment. However, the results from single-factor treatments were overall not found additive for the two-factor treatment, and of the mentioned accessions only two were found in the top for GPH under either of the single-factor treatments and the two-factor treatment. Considering more accessions than the top three to five best ones, though revealed broader overlap of accessions producing high GPH under the two-factor treatment and either of or both of the single-factors suggesting that high performance under a single-factor treatment can be beneficial in the two-factor treatment.

Under elevated [O_3], several accessions decreased less than the averaged 11 %, and 14 accessions increased > 11 % with regard to GPH. Cultivar variation to [O_3] have previously been reported in grain yield of soybean by Betzelberger et al. (2010) (with [O_3] applied eight hours a day at 40-150 ppb) and for the set of present accessions in Ingvordsen *et al*. (2014). Two old cultivars (Pallas and Juli) and an early modern cultivar (1978; Agneta) showed highest increased GPH under elevated [O_3] and Agneta also ranked one with regard to GPH under the [O_3] treatment of all 108 accessions.

Conclusions

The massive variation in protein response to the applied climate treatments, emphasize that the phenotypic differences should be exploited in breeding programs for abiotic stress tolerance. Likely, a cascade of different genes encodes the different responses. Here, mining the genome with GWAS (Genome Wide Association Studies) could help identifying some of the underlying genes, and the link between these DNA markers and phenotypes could facilitate the breeding process. Additionally, the identification of suitable genetic resources should be performed under treatments of combined climatic factors, since the effects from the single-factors were found rarely to be additive. The overall decreased GPH in the most realistic climate treatment, where [CO_2] and temperature were elevated simultaneously, emphasizes the need to explore and exploit genotypes to secure plant protein production under future climate conditions.

Supplementary data

Supplementary data are available at JXB online.

Supplementary Table S1. The 108 spring barley accessions included in present study with gene bank number, accession type, row type, origin country/breeding country, collecting date/release year, location/breeder institute, pedigree and marking of the 17 individual included in the chemical analysis.

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Table 1. Overview of the accessions included. Old cultivars before 1975; modern cultivars after 1975.

	Landraces		Old cultivars		Modern cultivars[a]	
	2 rowed	6 rowed	2 rowed	6 rowed	2 rowed	6 rowed
Denmark	1	2	6	1	23	
Sweden	1	1	7	2	2	1
Finland[b]	2	5	1	4	2	6
Norway	1		1	3		4
Europe[c]	5	7			7	
non-Europe	1	4				
unknown	1	5				

[a]including breeder-lines

[b]two landraces segregated either as 2 or 6 rowed and has not been included

[c]not including Scandinavian but Faroe Islands

Table 2. Experimental levels of manipulated climatic factors of applied treatments. Set points were; temperature (tmp): 19/12 °C (day/night) or 24/17 °C; [CO_2] (CO_2): 385 ppm or 700 ppm; [O_3] (O_3): 100-150 ppb; relative humidity 55/70 % day/night.

	tmp day/night	[CO_2] (constant)	[O_3] (constant)	humidity day/night
ambient	18.9±1.2/11.8±0.8	448.5±81.1	1.40±1.4	55.7±2.5/69.9±1.5
+CO_2	19.0±1.2/12.5±2.1	684.7±41.1	0.98±1.7	55.3±5.1/69.4±5.9
+tmp	23.9±1.4/16.8±0.8	448.4±74.4	1.90±1.2	55.9±2.8/69.8±1.6
+tmp & CO_2	23.8±1.3/16.9±0.9	688.3±38.2	1.50±1.4	56.0±2.9/69.8±1.8
+O_3	18.9±1.2/11.9±1.0	443.1±67.5	121.1±32.8	55.7±2.4/69.8±1.7

Table 3. Overall averaged parameters for the 108 barley accessions cultivated under future levels of carbon dioxide (+CO_2), ozone (+O_3), temperature (+tmp) and under the two-factor treatment (+tmp & CO_2) as well as under ambient (amb). * specifies significant difference from the ambient treatment determined by t-test. Grain yield per plant and grain number per plant are from Ingvordsen *et al*.(Ingvordsen *et al.*, 2014)

	amb	+tmp & CO_2	+ CO_2	+O_3	+tmp
Grain yield per plant (g)	6.85±1.29	4.92±1.18***	8.02±1.94***	5.82±1.38***	3.08±1.13***
% different from ambient		*-28.12*	*17.10*	*-15.10*	*-54.98*
Grain number per plant (#)	128.02±31.2	100.01±25.3***	149.93±17.1***	122.21±31.9	68.77±24.1***
% different from ambient		*-21.88*	*17.11*	*-4.54*	*-46.54*
Grain protein concentration (%)	13.97±1.82	15.06±1.97***	13.33±1.91*	14.76±1.96**	18.03±2.18***
% different from ambient		*7.86*	*-4.85*	*5.68*	*29.11*
Grain protein/grain (mg)	7.62±1.42	7.49±1.32	7.24±1.66	7.09±1.35**	8.14±1.62*
% different from ambient		*-1.63*	*-4.87*	*-6.84*	*6.82*
Grain protein harvested per plant (g)	0.95±0.20	0.74±0.19***	1.07±0.31**	0.85±0.18***	0.55±0.20***
% different from ambient		*-22.53*	*12.46*	*-11.19*	*-42.26*

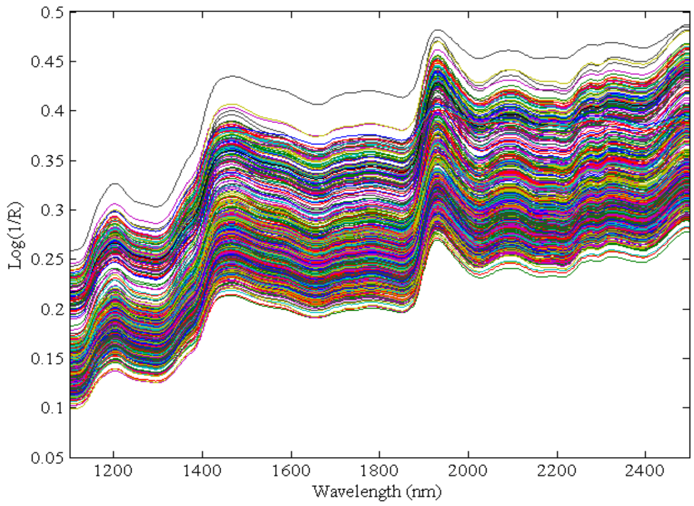

Fig. 1. Raw NIR spectra (1100 to 2498 nm) of all accessions.

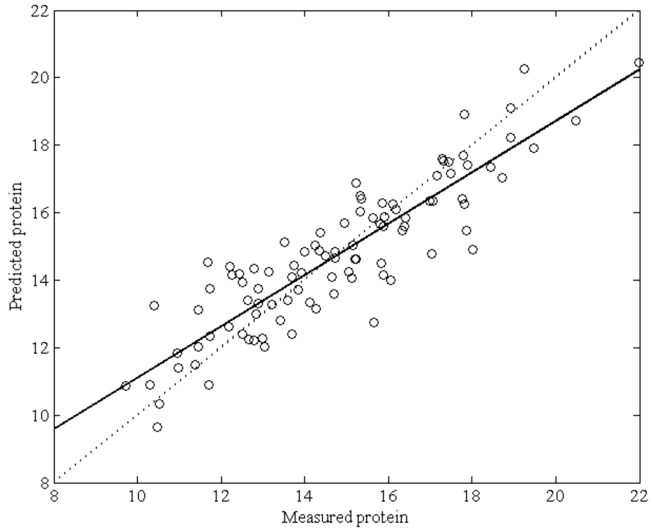

Fig. 2. Concentration of protein predicted using PLSR model vs. measured protein concentration. Full line indicate best fit with R^2=0.8 and RMSECV=1.3392. Dotted line has R^2=1

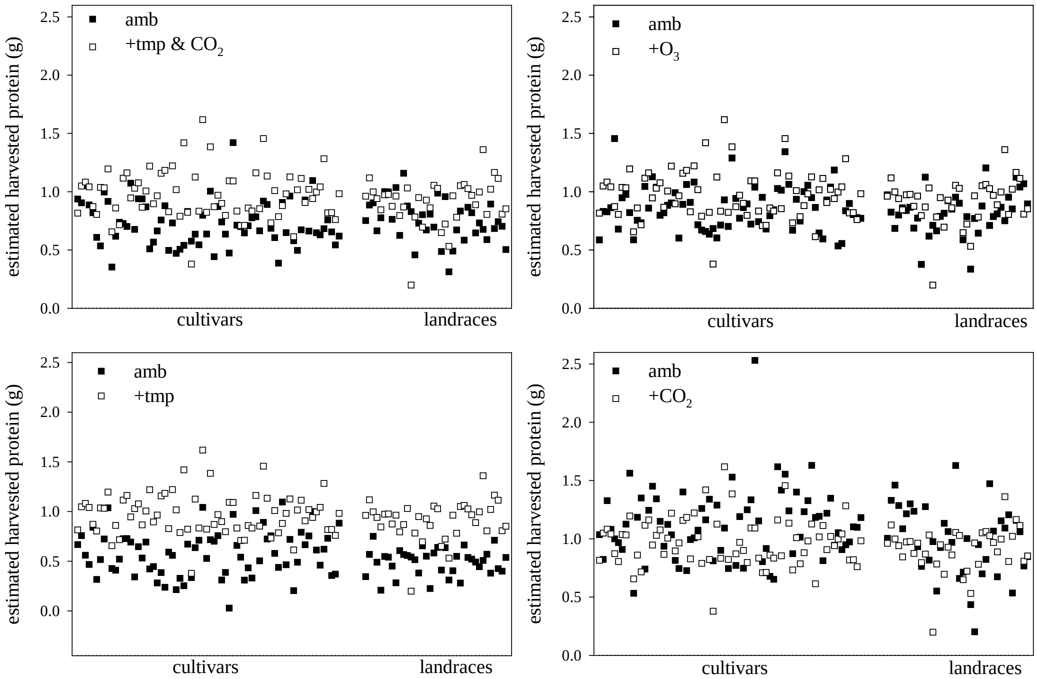

Fig. 3. Grain protein harvested (g/plant) of the 108 accessions in the five climate treatments (open) and ambient (closed). The order of accessions, left to right, is as in S1.

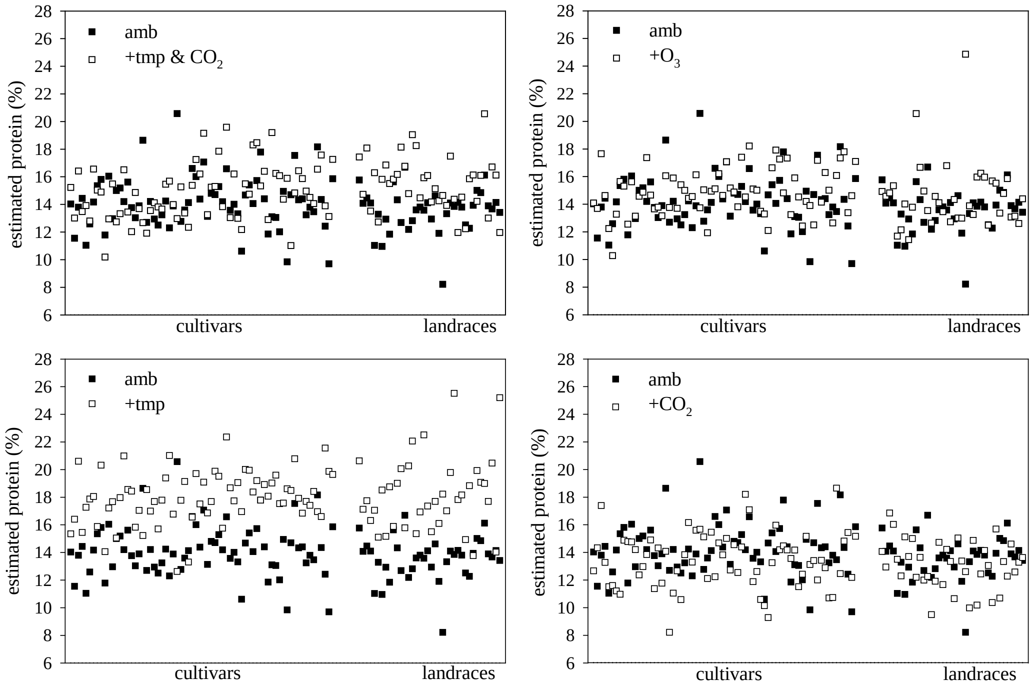

Figure 4. Grain protein concentration (%) of the 108 accessions in the five climate treatments (open) and ambient (closed). The order of accessions, left to right, is as in S1.